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Abstract: OBJECTIVE: To evaluate a novel device for its efficacy in removing experimental biofilm from root surfaces and its potential for concomitantly removing/roughening the surface substance. **METHODS AND MATERIALS:** A novel acrylic rotary device (biofilm remover, BR) was tested in vitro in three experiments: surface loss, surface roughness [positive controls: Periojet (PS) and Proxoshape (PR)] and biofilm removal [positive controls: ultrasonic (US) and PS]. Surface loss/surface roughness was evaluated for dentin samples instrumented for three 20 s periods. The calcium removed during instrumentation was analysed after each interval and cumulatively, using atomic absorption spectrophotometry (AAS). Surface roughness was measured using profilometric analysis. Biofilm removal was evaluated on dentin specimens coated with a 64.5 h 6-species in vitro formed biofilm, after one 20 s treatment. Surface loss was analysed using anova with Scheffé post hoc test, and surface roughness/biofilm removal was analysed using Mann-Whitney test (all $P < 0.05$). **RESULTS:** Significantly less substance loss [g (± 1 SD)] was observed with the novel device at all time points, both interval and cumulative (1.0 (± 0.5) versus 9.3 (± 3.2) PS and 9.9 (± 1.9) PR at 60 s). Surface roughness [μ m (95% CI)] was significantly lower for BR than for PS and PR [0.00 (-0.01, 0.08) 0.20 (0.16, 0.27) and 0.21 (0.19, 0.24) at 60 s]. Significantly less biofilm bacteria remained after treatment with both BR 4.5 (-0.1, 16.2) and US 1.9 (-0.2, 14.3), compared to PS 52 (27.9, 82.1). **CONCLUSIONS:** The novel biofilm remover was less damaging to dentin surfaces, while removing biofilm at least as effectively as devices used in this study.

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In Vitro Evaluation of a Novel Biofilm Remover

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Abstract

Objective: To evaluate a novel device for its efficacy in removing experimental biofilm from root surfaces and its potential for concomitantly removing/roughening the surface substance.

Methods and Materials: A novel acrylic rotary device (biofilm remover, BR) was tested *in vitro* in 3 experiments: surface loss, surface roughness (positive controls: PerioSet (PS) and Proxoshape (PR)) and biofilm removal (positive controls: ultrasonic (US) and PS). Surface loss/surface roughness were evaluated for dentin samples instrumented for three 20s periods. The calcium removed during instrumentation was analysed after each interval, and cumulatively, using atomic absorption spectrophotometry (AAS). Surface roughness was measured using profilometric analysis. Biofilm removal was evaluated on dentin specimens coated with a 64.5h 6-species *in vitro* formed biofilm, after one 20s treatment. Surface loss was analysed using ANOVA with Scheffé post hoc test and surface roughness/biofilm removal was analysed using Mann-Whitney-Test (all $p \leq 0.05$).

Results: Significantly less substance loss (μg (± 1 SD)) was observed with the novel device at all time points, both interval and cumulative ($1.0 (\pm 0.5)$ vs $9.3 (\pm 3.2)$ PS and $9.9 (\pm 1.9)$ PR at 60s). Surface roughness (μm (95% CI)) was significantly lower for BR than for PS and PR ($0.00 (-0.01, 0.08)$ $0.20 (0.16, 0.27)$ and $0.21 (0.19, 0.24)$ at 60s). Significantly less biofilm bacteria remained after treatment with both BR $4.5 (-0.1, 16.2)$ and US $1.9 (-0.2, 14.3)$, compared to PS $52 (27.9, 82.1)$.

Conclusions: The novel biofilm remover was less damaging to dentin surfaces, while removing biofilm at least as effectively as devices used in this study.

Clinical Relevance

Scientific rationale for the study: To date, no instruments for periodontal debridement are available that do not concomitantly remove root substance, which over repeated use may lead to iatrogenic root defects. A novel biofilm remover, specifically designed to debride without harming the root surface, was tested for safety and efficacy.

Principal findings: *In vitro* experiments studying surface loss, surface roughness and biofilm removal showed the novel biofilm remover to be as effective as an ultrasonic instrument in removing biofilm, while removing only negligible dentin, as compared to rotating or oscillating root planing instruments.

Practical implications: The novel biofilm remover may be a valuable addition to the SPT armamentarium. As effective as an ultrasonic instrument for biofilm debridement and significantly less harmful than the finest root planing bur (15 µm) or oscillating tip on dentin, the novel biofilm remover shows promise for clinical use. *In vivo* testing, however, is still needed to determine if this promise will be fulfilled under practical application in residual pockets and other hard to reach niches.

Conflict of Interest Statement: The novel biofilm remover has been developed and patented by **Prof. Dr. P.R. Schmidlin**, University of Zurich, in cooperation with Kerr Hawe SA, Bioggio, Switzerland. The prototype biofilm remover inserts were provided free of charge from Kerr Hawe SA. Funding for the study was provided solely by the University of Zurich Clinic for Preventive Dentistry, Cariology, and Periodontology.

Introduction

Some persisting pocketing is common after initial cause related therapy (ICRT) (1-3). These residual problems are in turn the targets of supportive periodontal therapy (SPT). Depending upon the depth of the residual pockets and the anatomical features that prohibit adequate biofilm removal by the patient, these residual loci of infection may continue to trigger the cascade of biological host responses whose clinical outcome is on-going periodontal disease. The goal of SPT is to debride these niches of subgingival biofilm, mostly at short intervals (2 - 3 months), in order to prevent further disease progression. The ultimate challenge is to do so without undue and unnecessary removal of root substance, especially when performing repeated subgingival instrumentation (4).

Studies have shown that the most common treatment methods of scaling and root planing (SC/RP), ultrasonic instrumentation and the use of rotary instruments have not met this challenge to date (5-10). In the 2002 review by Cobb, evidence is provided showing that scaling/root planing and the use of ultrasonic instruments remove/miss similar amounts of calculus and biofilm, with the ultrasonics providing these results while consuming less time. In later studies (11), which also examined rotary instruments, these instruments provided a visually smooth surface with less surface roughness than Gracey curettes. However, in none of these studies was the root surface left undisturbed. Cementum removal and dentinal loss are almost inevitable by-products of plaque and calculus removal. As a consequence, SPT patients are at risk of losing significant amounts of tooth structure when conscientiously complying with a strict regime of SPT treatments. As effective biofilm removal is the main objective of SPT, treatment methods still need to be established

that provide the necessary soft debridement, without negative side effects to tooth and periodontal structures.

To this end, a newly developed device composed of a biocompatible epoxy with helical flute form, designed to flex and wear itself down when coming in contact with dental hard tissue, was evaluated in this 3-part *in vitro* study for its efficacy in removing adhering experimental biofilm from root surfaces, its potential for less aggressive concomitant removal of root substance, i.e. dentin, and its ability to debride plaque without roughening the root surfaces. The hypothesis tested was that this biofilm remover (BR) would remove biofilm from dentin samples as efficaciously as an ultrasonic instrument (US) or rotary diamond-coated instrument (PS), while at the same time removing less dentin, and causing less surface roughness, than either a rotary (PS) or oscillating (PR) diamond-coated instrument.

Materials and Methods

Part 1- Evaluation of tooth substance loss

Twenty-eight specimens from extracted human premolars stored in physiological saline at 4°C were prepared for this experiment. These teeth had been collected and pooled, anonymously, after cause-related treatment at the dental school. As such, our Medical Ethical board states that the performed research does not fall under the regulations of the Act on Medical Research Involving Human Subjects (METc 2009.305). A written informed consent was therefore not compulsory. Nevertheless, patients were informed about general research purposes and had given informed consent, for future research use of their extracted teeth.

Roots were first separated from the tooth crowns and ground in half by a rotating sandpaper (180 grit silicon carbide sandpaper, Struers GmbH, Birmensdorf, Switzerland) device at 150 rev./min. (Planopol-2®, Struers,). They were then further divided into 6 by 10 mm specimens, using a diamond-coated disc (918P Ø 220 mm, Komet mounted on Mandrel 303, Komet, Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) in a slow counter-angle hand piece (Micro Mega, Genève-Acacias, Switzerland) under constant water-cooling. These specimens were then finished on a rotating device (Planopol-2®, Struers, Merck (Switzerland) AG, Dietikon, Switzerland) using sandpaper with a grit size of 1000 grit (Struers). This polishing procedure ensured a comparable surface roughness and served as baseline before instrumentation.

Samples were then embedded in a chemically curing acrylic resin (Paladur[®], Heraeus Kulzer GmbH, Wehrheim, Germany), with an upper access window of 3 by 5 mm, and then glued on to roughened SEM mounts (Baltec AG, Blazers, Liechtenstein) with superglue (Renford Sekundenkleber Nr. 1733, Dentex AG, Zurich, Switzerland). The prepared specimens were stored in distilled water before use at 22 °C.

Twenty-seven of these samples were then randomly assigned to one of three treatment groups (n=9): diamond rotating bur (PS; 15 µm; Periojet RA 515, Intensiv SA, Montagnola, Switzerland), diamond-coated oscillating file (PR; 15 µm; Proxoshape Flexible PS3L, Intensiv SA, Montagnola, Switzerland), and novel rotating device (BR; Biofilm Remover prototype, Kerr Hawe, Bioggio, Switzerland) (Fig. 1). For this purpose, specimens were fixed onto a pressure scale (Fig. 2), for consecutive instrumentation over the entire window area during three 20 s intervals, by one operator (DH) at a pressure of 50 g; speeds of 100,000 rpm (PS), 750 rpm (BR) and 3000 rpm (PR); and an angulation of 0°. Each instrument was cooled with water during instrumentation. The coolant water from the 3 active treatment groups and the rinse water from the control group was carefully collected in acrylic cups (Fig. 2) and analysed after each interval using High Resolution Continuum Source AAS (atomic absorption spectrophotometry) at 422.7 nm to determine the amount of calcium lost at each time interval to the treatment procedure: The collected sample solutions were diluted with 10 ml of hydrochloric acid (2 M), then placed in an ultrasonic bath for five minutes to dissolve the insoluble dentin particles and to avoid precipitation. Demineralized water was added to an end volume of 50 ml; 2 ml of the solution was extracted and 4.6 ml demineralized water, as well as 3.4 ml SrCl₃

complimented the solution for AAS analysis (ContrAA 300, AJZ Engineering GmbH, Jena, Germany). Calcium from the dissolved dentin in solution was determined in µg.

Part 2 – Evaluation of surface roughness

The twenty-seven samples instrumented above, were evaluated for surface roughness after each 20 s treatment phase; the 28th specimen, reserved for baseline determination for this part of the experiment, was measured just once. Measurements were made with a precision profilometer (Form Talysurf-50, Rank Taylor Hobson Ltd., Leicester, UK). The root surface was traced with a stylus with a 90° angle (WIB 90, ELYT Special) and 50 mm length. The vertical displacements were electronically converted using a specially designed software (Ultra, Version 5.14.9.70, Rank Taylor Hobson Ltd., Leicester, UK) to calculate the arithmetic average of surface roughness (Ra). Measurements were taken vertically and horizontally to the root axis, with a 1 mm boarder at each edge and 0.5 mm between each measurement line, beginning 1 mm coronal from the apical extent. Six measurements per root specimen were recorded. The profilometric readings were repeated three times for each experimental surface. By measuring the horizontal and vertical paths, roughness resulting from a free-hand instrumentation of the surfaces was most likely to be captured.

SEM Analysis

One specimen from each of the instrumentation groups and after each 20 s treatment phase, plus the baseline specimen, was also examined under a scanning electron microscope (Supra 50V, Carl Zeiss AG, Oberkochen, Germany; gold-sputtered (16 nm) with a sputtering device (Sputter SCD 030, Baltec AG, Balzers, Liechtenstein)

for structural damage such as scratches, gouges and cracks.

Part 3 - Biofilm removal

Thirty-six hydroxyapatite discs (Ø 10.6 mm; Dense Hydroxylapatite Discs, Art. 071102, Clarkson Chromatography Products Inc., South Williamsport, USA) were embedded in a silicone-based impression material (President light-body, Coltène/Whaledent, Altstätten, Switzerland) to ensure that biofilm formation only occurred on the test surface. Pre-tests with food colouring (1% solution; E131, Food Blue 5, Merck (Switzerland) AG, Dietikon, Switzerland) were performed to prove the impermeability of the impression material as an embedding substance.

Streptococcus oralis (OMZ 918), *Veillonella dispar* (OMZ 493), *Fusobacterium nucleatum* (OMZ 598), *Streptococcus mutans* (OMZ 607), *Actinomyces oris* (OMZ 745) and *Candida albicans* (OMZ 110) were used as inocula for biofilm formation (12)- (13). Biofilms were grown in 12-well polystyrene cell culture plates (Nunc, Roskilde, Denmark). In brief, discs were preconditioned (pellicle-coated) in 1.5 ml processed whole un-stimulated pooled saliva and incubated for 4 hours at room temperature. A mix of 14 ml medium 1 (FUM + glucose) plus 6 ml of processed saliva was made and equilibrated for 45min (37 °C, anerobic). To start a biofilm experiment, the discs were covered with 1.6 ml of growth medium (saliva/mFUM) and 200 µl of a cell suspension prepared from equal volumes and densities of each strain. mFUM corresponds to a well-established tryptone-yeast-based broth medium designated as FUM (14) and modified by supplementing 67 mM Sørensen's buffer (final pH 7.2). The carbohydrate concentration in mFUM was 0.3% (w/v), and consisted of glucose

for the first 16.5 h and from then on of a 1:1 (w/w) mixture of glucose and sucrose (see below). Biofilms were incubated anaerobically at 37 °C for 64.5 h. After inoculation, the discs remained in the feeding solution containing 0.3% glucose for 45 min. Thereafter, they were subjected to three consecutive 1 min washes in 2 ml 0.9% NaCl to remove growth medium and free floating cells but not bacteria adhering firmly to the discs. The biofilms were then further incubated in new wells containing 1 ml of saliva and no mFUM. Only after 16.5, 20.5, 24.5, 40.5, 44.5 and 48.5 h biofilms were pulse-fed by transferring the discs for 45 min into 30% saliva / 70% mFUM with 0.15% glucose and 0.15% sucrose. They were then washed as described above and re-incubated in saliva. Fresh saliva was provided after 16.5 and 40.5 h. After 64.5 h the biofilm-covered discs were treated, then analysed, using the protocol below.

Samples were randomly assigned to one of four treatment groups (n=9): diamond rotating bur (PS; 15 µm; Periojet RA 515, Intensiv SA, Montagnola, Switzerland), ultrasonic cleaning device (US; Perioscan, Tip 4PS, Sirona GmbH, Blensheim, Germany), novel rotating device (BR; Biofilm Remover prototype, Kerr Hawe, Bioggio, Switzerland) (Fig. 1) and a control group (physiological saline rinse only at each interval). The discs, having been carefully removed from the embedding material with sterile college pliers (P15 Tweezer, Deppeler SA, Switzerland), were held on their rims with the pliers and the test surfaces instrumented by the same operator (DH) as described above for 20s. Again, a 50 g pressure and angulation of 0° was employed, as calibrated in the substance loss trial. The instrument speed for PS was 100,000 rpm and 750 rpm for the BR, respectively. The ultrasonic power setting used was Perio 1 (at 75%). Each instrument was cooled with water, as under clinical conditions. Each disc in the BR and PS groups was treated with a factory new instrument. After each treatment, discs were rinsed by being double-dipped

sequentially in 3 x 2-ml portions of fresh physiological saline. The discs in the control group did not undergo treatment and were dipped 3 times in 2 ml of physiological saline.

The discs from the 3 treatment groups were transferred immediately after their treatment (and the control group after being rinsed) to individual Falcon tubes filled with 1000 µl physiological saline and residual bacterial subsequently harvested as follows: each disc was placed in a sterile plastic Petri dish. Four-hundred µl physiological saline was taken from the Falcon tube and set to the cover-edge of the Petri dish. The discs were covered with 100 µl physiological saline taken from the cover edge and scraped by use of a sterile plastic scaler (Implant Deplaquer, Kerr, Bioggio, Switzerland). This procedure was repeated four times. The scraped discs and the Petri plate were rinsed with the remaining liquid from the Falcon tube (2x 300 µl). The pooled washings (final volume of 1000 µl) were collected in a new Falcon tube. The cell suspensions were then sonified for 5 sec at 30 W and room temperature. The harvested, suspended biofilms were diluted (undiluted, 10^{-2} , 10^{-4}) and vigorously vortexed for 5 seconds. Using a spiral plater, the diluted and undiluted suspensions were spirally plated onto Columbia Blood Agar Base (Difco Laboratories, Inc, Detroit, MI, USA) with 5% human blood. After 72 hours of anaerobic incubation at 37 °C, the colony forming units (CFU) were counted under a stereomicroscope. The total CFU were determined on Columbia blood agar.

Statistical Analysis

All data were tested for normal distribution using the Kolmogorov-Smirnov test. The data for surface loss were compared using ANOVA with Scheffé post-hoc test. The data for surface roughness and biofilm removal were analysed using Kruskal–Wallis one-way test of variance followed by Mann-Whitney test for individual comparisons. Significance for all tests was set at 95% ($p \leq 0.05$; SPSS Statistics Version 20, IBM, USA).

Results

Substance loss

The calcium loss (μg) from the dentin samples, individually and cumulatively at each of three 20 s intervals, is presented in Table 1. Calcium abraded from the root surfaces during instrumentation was fairly constant at each of the 20 s time intervals for all instruments tested. There were, however, significant inter-group differences between the BR group and the PS and PR groups. No significant difference was found between the two diamond-coated instrument groups, PS and PR.

Surface roughness

The surface roughness measurements (Ra-values; median of horizontal and vertical measurement changes in μm) are shown in Table 2. BR created minimal roughness (0.00 (-0.02, 0.07)), that remained essentially unchanged over the three 20 s test periods. PS created neither significantly more roughness than BR, nor significantly less roughness than PR at 20 s. By 60 s, PS and PR created similar distinguishable roughness of 0.20 (0.16, 0.27) and 0.21 (0.19, 0.24), respectively.

SEM analysis

Visual comparison of the untreated (baseline), and treated (BR, PS, PR) surfaces after three 20 s instrumentation applications, are presented in Figure 3. Different roughness patterns are discernible for the diamond-coated instruments PS and PR, while BR does not appear to have affected the surface structure, when compared to baseline.

Biofilm removal

Mean CFUs remaining after BR, PS and US instrumentation are shown in Table 3. Best results in biofilm removal were obtained through use of US and BR. The rotating diamond-coated PS removed significantly less biofilm than the steel US tip or the acrylic BR tip.

Discussion

The results presented support the initial hypothesis that the biofilm remover (BR) would remove biofilm from dentin samples as efficaciously as an ultrasonic instrument (US), although the efficacy of the rotary instrument (PS) was significantly lower than anticipated. With regard to dentin removal, as hypothesized, the biofilm remover removed significantly less tooth substance than either the rotary (PS) or oscillating (PR) diamond-coated instruments. Likewise, BR caused significantly less surface roughness than PS or PR, which were comparable to each other.

The study design used was intentionally divided into 3 parts. Parts one and two, dealing with potential substance loss and damage potential, tested 2 diamond-coated instruments (one rotating and one oscillating) against the novel biofilm remover. Both of the diamond-coated instruments represented root planers. Ultrasonic devices, on the other hand, are not root planing instruments *per se*. Although not definitively proven, they have been shown in some studies to cause dental hard tissue removal (8, 15, 16). Since our intention was to test instruments that may reach into residual periodontal pockets and remove biofilm for SPT, with a minimum loss of tooth structure (hence the choice of 15 μm diamond-coated instruments), it was decided to exclude the ultrasonic device from this experiment. However, in hindsight, this may be a limitation in evaluating our results. Previous studies measuring tooth substance loss evaluated defect depth in μm , whereas the current study looked at surface loss weight in μg , and as such the collective results are difficult to compare. Conversely, since the two diamond-coated instruments were similar in grit, it was deemed redundant to use both in the biofilm removal experiment, where the efficacy of an

ultrasonic device and a diamond-coated instrument compared to the biofilm remover was tested.

The methods employed in this study (standardized pressure, angulation and time units) were chosen to conform to previously published studies (17-19). In a recent non-conforming study, 500g of pressure was used in order to “ensure substance removal sufficient for measurement using digital calipers”. This working pressure is considerably greater than the maximum of 0.05 N – 1.0 N recommended in the aforementioned studies (16). The need to use clinically excessive force is not necessary when measuring substance loss with atomic absorption spectrophotometry (AAS) and the current study thereby retains a semblance of clinical applicability, which may be helpful when evaluation the results for potential use *in vivo*.

In a 1992 systematic review of studies looking at the periodontal response to non-surgical therapy (7), Greenstein laid out the parameters and supporting research for bacteria in a biofilm as the trigger for a host inflammation response, the limitations of supragingival biofilm removal to resolve a subgingival infection and the ability of these biofilms to re-establish themselves within 60 days of SPT. The resulting need to remove subgingival biofilm on a regular basis, to maintain the therapeutic effects of ICRT, was shown here and in later publications (10). However, in spite of compliance with SPT regimes, residual and relapsed pocketing after ICRT has been shown in a number of long-term studies (> 10 years) to develop in risk patients and at certain sites even when compliance with SPT is given (20). The at-risk sites are especially those that are ≥ 6 mm in dept (21, 22), molars and premolars (21), furcations and root concavities (23); all parameters that hinder adequate plaque

removal. According to the 5th European Workshop on Periodontology (22), this constitutes periodontal disease progression (also as a factor of a suboptimal ICRT outcome), whose treatment has yet to be satisfactorily resolved.

The often-cited reasons for clinicians to use diamond-coated instruments are that they are faster, less taxing on the clinician and provide a smooth or smoother root surface than do hand instruments when used by less-skilled clinicians. Scanning electron microscope studies concentrating on the quality of the instrumented surface achieved seem to support their use (11, 24, 25). While the findings from these studies have been mostly qualitative (SEM analysis) or semi-quantitative (RLTSI, Roughness and Loss of Tooth Substance Index), one study (11) also provided quantitative (profilometric) data showing no significant difference in surface roughness between a diamond-coated bur and Gracey curettes.

Atomic absorption spectrometry (AAS) provides quantitative data on surface substance loss, and is sensitive enough to detect small amounts down to a lower limit of 0.08 µg/ml. This standardized test has been primarily used in demineralization studies. However, two recent studies (26, 27), have also shown AAS to be an effective and quantifiable mean of measuring minimal amounts of dentin loss due to periodontal instrumentation.

Only one study found (28) also provided their data in the form of weight loss after instrumentation. An electronic analytic balance, with a measurement precision of ± 100 µg was used. The results showed an average surface loss of 0.4 – 0.5 µg surface loss for all of the instruments tested. Two factors inhibit a direct comparison of this to the current study: where the 2005 study used extracted teeth that had not

been periodontally treated (presumably orthodontic extractions), the substance removed must have been cementum, which is lighter than dentin. Second, different ultrasonic and oscillating instruments were used in both studies, making a direct comparison impossible. A general trend, however, is discernible: all of the ultrasonic, rotating and oscillating instruments in both studies removed considerably more tooth substance than did the biofilm remover in this study.

Not surprisingly, surface roughness was similar for the 2 diamond-coated instruments after 60 s. Since both instruments were coated with the same grit size, their mechanisms of action, PS rotating and PR oscillating, may account for the slight variation at 20 s and 40 s. However this difference did not reach the level of statistical significance. By the 60 s mark, due to the constant movement of the instruments over the test surfaces in all directions, the mechanism of action was probably no longer relevant to the creation of a roughened root surface.

The fact that the readings for surface roughness between PS and PR were so similar may also be a further confirmation that both instruments were used with the same consistent pressure. In contrast, BR, with the same pressure and instrumentation time, produced negligible roughness. This, in combination with the effective biofilm removal achieved, shows not only its potential for removing the predictor of continued periodontal disease but also avoiding roughening of the root surface, which is believed to enhance new plaque adherence (10). Further use of a non-abrading BR may help avoid, cumulatively over time, denuding of the cementum layer and causing dentinal defects as the result of necessary instrumentation during SPT.

Although the prototype biofilm remover (Fig. 1) was designed to access deep infrabony defects, this *in vitro* study to test safety and efficacy was undertaken on flat dentin specimens. While this represents a limitation when interpreting the results, the fact remains that where it came in contact with tooth structure, it was equal to an ultrasonic (and superior to a rotating diamond coated) instrument in removing biofilm. It must also be assumed that these other instruments operated under a similar limitation (indeterminate degree of contact) when applied to an *in vivo* site, where past studies have shown their level of efficacy both through the proxy parameters of decrease probing pocket depth and bleeding on probing, as well as the superficial physical characteristics of surface roughness and depth of defects after extraction of experimentally treated, but not-rational-to-keep periodontally involved teeth (16, 24, 25, 29).

Despite this testing limitation, it must be remembered that deep infrabony pockets, furcations and root concavities remain largely inaccessible for SPT. Instruments capable of debridement through adequate surface contact in these areas are still being sought. The biofilm remover may fill this unmet need, though further testing is necessary. Where the biofilm remover appears superior to currently available instruments used in SPT is in its ability to debride without significant concomitant loss of dentin. In that light, the results of this study are indeed promising.

Conclusions

Within the limitations of this *in vitro* study, the biofilm remover has proved to be an effective means of removing biofilm from dentin surfaces with significantly less loss of substance or increase in surface roughness than the standard treatment modalities of ultrasonic, rotating and oscillating instruments. Further studies are needed to determine its efficacy *in vivo*.

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Table 1. Mean surface loss over time ($\mu\text{g} \pm 1 \text{ SD}$), after each treatment interval and cumulatively

Treatment Time	BR	PS	PR
1 st 20s interval	0.5 (± 0.2) ^A	2.8 (± 1.6) ^B	3.1 (± 0.9) ^B
2 nd 20s interval	0.3 (± 0.1) ^A	3.2 (± 1.1) ^B	3.6 (± 0.7) ^B
3 rd 20s interval	0.2 (± 0.1) ^A	3.3 (± 0.9) ^B	3.3 (± 0.6) ^B
Treatment Time	BR	PS	PR
20s	0.5 (± 0.2) ^A	2.8 (± 1.6) ^B	3.1 (± 0.9) ^B
40s cumulative	0.8 (± 0.4) ^A	6.0 (± 2.4) ^B	6.6 (± 1.4) ^B
60s cumulative	1.0 (± 0.5) ^A	9.3 (± 3.2) ^B	9.9 (± 1.9) ^B

BR=Biofilm Remover, PS=PerioSet, PR=Proxoshape

Differing upper case letters (read horizontally) signify significant inter-group differences (ANOVA with Scheffé post-hoc test, $p \leq 0.05$)

Table 2. Median surface roughness over time (R_a in μm (95% CI))

Treatment Time	BR	PS	PR
20s	0.00 (-0.02, 0.07) ^A	0.11 (0.04, 0.18) ^{A,B}	0.14 (0.13, 0.15) ^B
40s cumulative	0.00 (-0.01, 0.08) ^A	0.14 (0.11, 0.26) ^B	0.20 (0.17, 0.27) ^B
60s cumulative	0.00 (-0.01, 0.08) ^A	0.20 (0.16, 0.27) ^B	0.21 (0.19, 0.24) ^B

BR=Biofilm Remover, PS=PerioSet, PR=Proxoshape

Differing upper case letters (read horizontally) signify significant inter-group differences (Kruskal-Wallis one-way test of variance, Man-Whitney test for individual comparisons, $p \leq 0.05$)

Table 3. CFUs (median $\times 10^5$ (95% CI)) after 20s treatment

BR	PS	US	Control
4.5 (-0.1, 16.2) ^A	52 (27.9, 82.1) ^B	1.9 (-0.2, 14.3) ^A	4900 (4395.3, 5842.2) ^C

BR=Biofilm Remover, PS=PerioSet, US=ultrasonic devise

Differing upper case letters (read horizontally) signify significant inter-group differences (Kruskal-Wallis one-way test of variance, Man-Whitney test for individual comparisons, $p \leq 0.05$)

Figure 1. Instruments used in the present study (from left to right): Biofilm Remover (BR), PerioSet (PS), Proxoshape (PR), PerioScan (US)

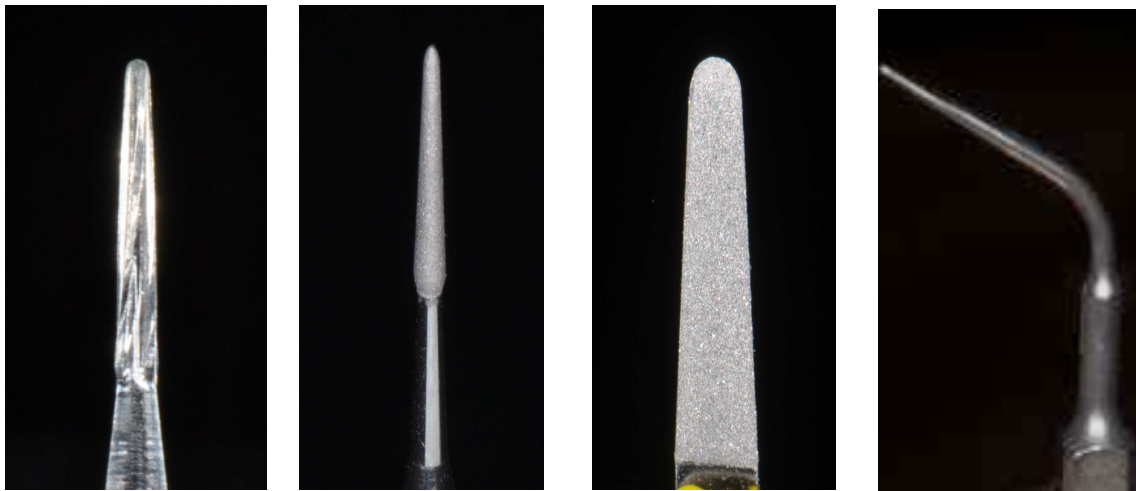


Figure 2. Determination of the tooth substance loss, i.e. calcium capture set-up, with standardized load control and liquid collection device.

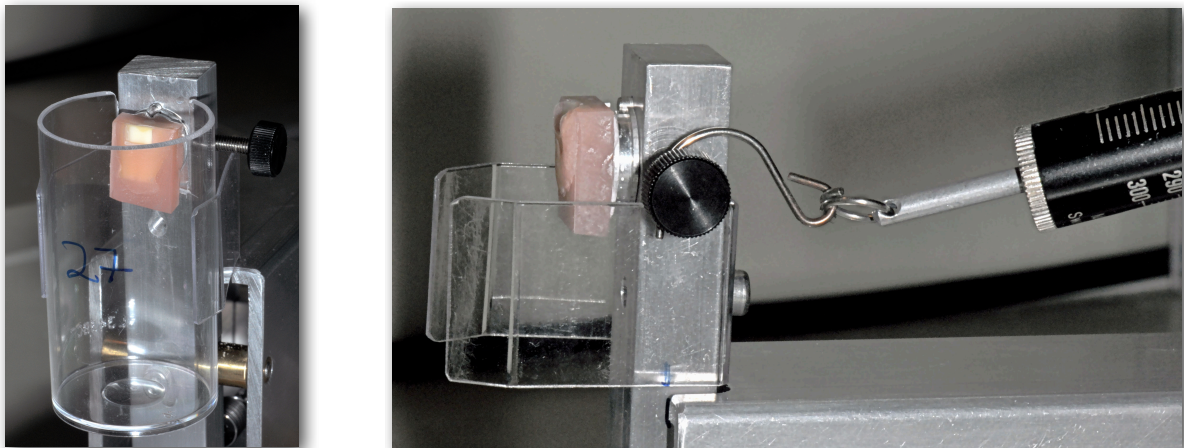


Figure 3. SEM images (500x magnification) at baseline and after 60 s treatment with Biofilm Remover (BR), Perioiset (PS) or Proxoshape (PR).

